Alcoholic Fermentation of Raw Sweet Potato by a Nonconventional Method Using Endomycopsis fibuligera *Glucoamylase Preparation*

INTRODUCTION

In recent years, alcoholic fermentation has received much attention as an alternative energy source. In conventional alcoholic fermentation from starchy materials, precooking is necessary for liquefaction and saccharification of the broth, which requires a large amount of heat energy—about 30–40% of all energy spent for alcohol production. Ueda and his co-workers[1,2] have attempted to produce ethanol from raw starch in a single-step process, which combines liquefaction, saccharification, and yeast fermentation without cooking and autoclaving by using glucoamylase preparation from *Aspergillus niger* in order to save the cost of energy consumption by cooking. Ueda[3] has also reported alcoholic fermentation of sweet potato without cooking by using *Rhizopus* glucoamylase preparation.

In the present communication, we report the effectiveness of alcoholic fermentation of sweet potato without cooking by using *Endomycopsis fibuligera* glucoamylase preparation.

MATERIALS AND METHODS

Materials

Sweet potato tubers were purchased from a local market. Glucoamylase preparation from *E. fibuligera* (IFO 0111) was obtained by batch cultures in 500-mL Erlenmeyer flasks containing 100 mL of liquid medium (1% soluble starch and 0.5% yeast extract), pH 6.0, on a reciprocal shaker with a frequency of about 150 cycles/min at 25°C for four days. The culture filtrate was treated with ammonium sulfate (0.8 saturation), kept overnight, and centrifuged. The precipitate was dissolved in water and then dialyzed against water for 48 h. The dialyzed solution was used as glucoamylase preparation. Cellulosine HC, which is a mixture of crude cellulase, hemicellulase, xylanase, pectinase, etc., was purchased from Ueda Chemical Industry Co. (Osaka, Japan). The yeast used was compressed baker's yeast (*Saccharomyces cerevisiae*) manufactured by Oriental Yeast Industry Co. (Osaka, Japan).

Enzyme Assay

Glucoamylase activity was assayed by a method previously reported[4] with only slight modification. The assay is based on determining the amount of glucose liberated in a reaction mixture of 5 mL of 1% boiled soluble starch solution, 1 mL 0.02*M* acetate buffer, pH 4.8, 1 mL deionized water, and 1 mL of suitably diluted enzyme solution in a final volume of 8 mL during 10 min incubation at 40°C by micro-Bertrand method. One unit of glucoamylase activity is that amount of enzyme which produces 1 mg glucose in 1 mL of the reaction mixture under these conditions.

Raw Starch Digestion

Raw starch digestion was tested as follows. The reaction mixture containing 50 mg of raw wheat starch (Wako Pure Chemicals Co., Japan), 0.5 mL of 0.5*M* sodium citrate-HCl buffer of the desired pH, 1 mL of deionized water, 1 mL of enzyme solution, and a few grains of thymol was incubated at 30°C with occasional shaking. At a suitable time interval, the reducing sugar liberated in 1 mL of the reaction mixture was determined by micro-Bertrand method and the hydrolysis percentage was calculated.

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Alcoholic Fermentation

The sweet potato tubers were mashed. Mashed sweet potato (50 g), *E. fibuligera* glucoamylase preparation (1000 units), cellulosine HC (100 mg), potassium pyrosulfite (50 mg), yeast (3 g), and tap water (100 mL) were put in a 300-mL Erlenmeyer flask and the broth was adjusted to pH 4.5 with 20% phosphoric acid. The flask contents were protected from the atmosphere by a tube containing concentrated sulfuric acid. This system can pass CO_2 but water vapor is adsorbed by the sulfuric acid so that the weight loss of the contents represents the formation of CO_2. The flask was incubated at 38°C with frequent shaking and weighed each day. At the end of the fermentation, the alcoholic content of the broth was determined with an alcohol hydrometer after distillation.[5] The total acidity of the broth was measured as the amount of 0.1*N* NaOH required to neutralize 10 mL of the broth.

Total Sugars of Sweet Potato or Fermented Broth

The total sugar of the sweet potato or fermented broth was determined by measuring the reducing sugar liberated by acid hydrolysis. One gram of mashed sweet potato, 10 mL of 25% HCl, and 100 mL of deionized water were heated for 3 h at 100°C. Then, the reducing sugar of the neutralized filtrate was determined by micro-Bertrand method. In the case of broth, 1 mL was hydrolyzed with 1 mL of 25% HCl and 8 mL of water for 3 h at 100°C.

RESULTS AND DISCUSSION

Raw starch digestion by *E. fibuligera* glucoamylase preparation at various pH is shown in Figure 1. It was found that the enzyme preparation digested raw starch optimally at pH 4.5. So this optimum pH for raw starch digestion by *E. fibuligera* glucoamylase preparation was similar to that (4.5) of *Rhizopus* glucoamylase preparation but different from that (3.5) of *A. niger* glucoamylase preparation.[3]

Raw starch digestion by different glucoamylase preparations was tested at their respective optimum pHs in order to compare their raw starch digestion capability. The results are shown in Table I. It is evident from this that the raw starch digestion capacities of these amylase preparations differed from each other. *E. fibuligera* or *Rhizopus* glucoamylase preparation was superior

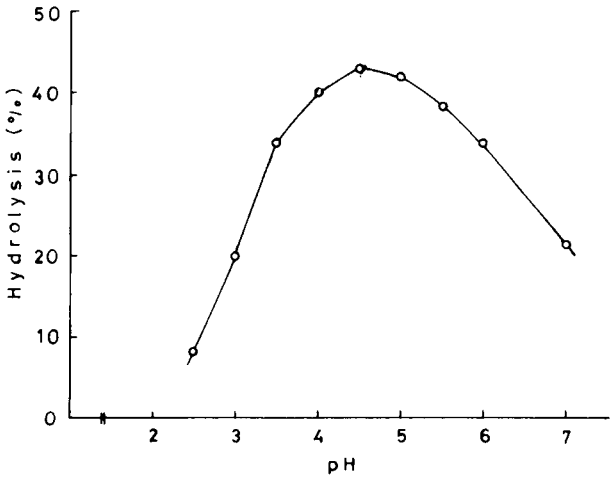

Fig. 1. Effect of pH on raw starch digestion by *E. fibuligera* glucoamylase preparation (1.2 units); reaction time is 18 h.

TABLE I
Comparison of Raw Starch Digestion by Various Glucoamylase Preparations

Enzyme preparation	Hydrolysis[a] (18 h) (%)
Aspergillus niger	
Glucuzyme GNL-2000 (Amano)	21.6
Sumizyme AN (Shin-Nihon)	39.3
NEO XL-128 (Nagase)	38.0
AMG (Novo)	37.5
Rhizopus sp.	
Glucuzyme GR-2 (Amano)	47.0
Endomycopsis fibuligera	
Glucoamylase preparation (laboratory)	47.0

[a]An amount of 2.07 units enzyme was used in every case. The pH of raw starch digestion was 3.5 in case of *A. niger* and 4.5 in two other cases.

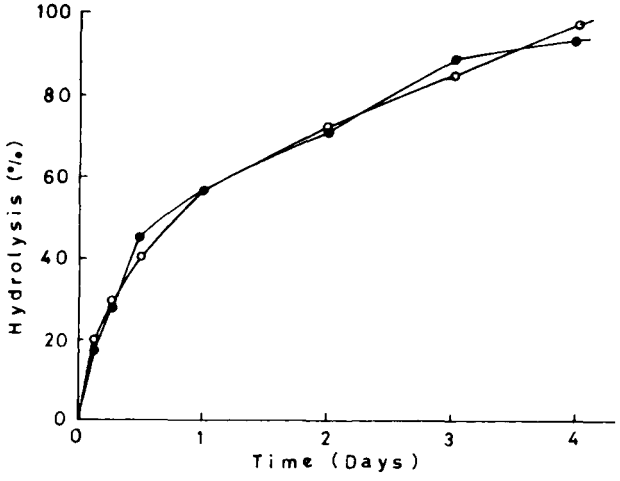

Fig. 2. Time course of raw starch digestion by *E. fibuligera* and *Rhizopus* glucoamylase preparations (1.2 units each): (○) *E. fibuligera* glucoamylase preparation and (●) *Rhizopus* glucoamylase preparation.

TABLE II
Effect of Potassium Pyrosulfite on Raw Starch Digestion by *E. fibuligera* Glucoamylase Preparation

Quantity (mg)	Hydrolysis[a] (16 h) (%)
0.0	40.75
0.4 (0.008%)	41.53
1.0 (0.04%)	38.75
2.0 (0.016%)	38.50

[a]The enzyme used was 1.74 units in every case.

to *A. niger* in this respect. A time course of raw starch digestion by *E. fibuligera* and *Rhizopus* glucoamylase preparations is shown in Figure 2; this also indicates that the two enzyme preparations were very similar to each other with respect to raw starch digestion ability.

The nonconventional fermentation (without precooking) faces a threat of contamination by foreign bacteria since there is no sterilization process. Ueda[3] reported that in order to prevent the contamination of the fermentation broth, 0.02% or more potassium pyrosulfite can be effectively used in case of alcoholic fermentation of sweet potato without cooking using *Rhizopus* glucoamylase preparation. We tried this to check whether potassium pyrosulfite has any effect on raw starch disgestion by *E. fibuligera* glucoamylase preparation; the results are presented in Table II. It was found that potassium pyrosulfite had little effect on raw starch digestion by this enzyme preparation. But, for alcoholic fermentation, 0.05% potassium pyrosulfite was found to have very good effect in combating contamination of the fermentation broth.

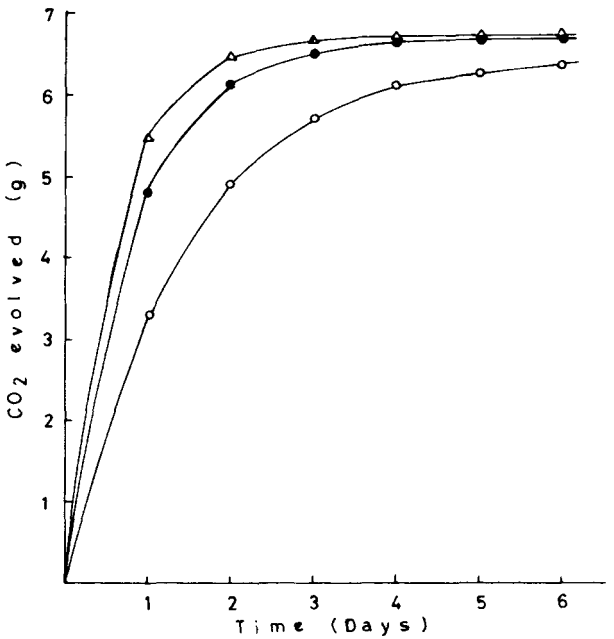

Fig. 3. Alcoholic fermentation of raw sweet potato (total sugar, 14.63 g) at three different temperatures: (○) 30°C, (●) 35°C, and (△) 38°C.

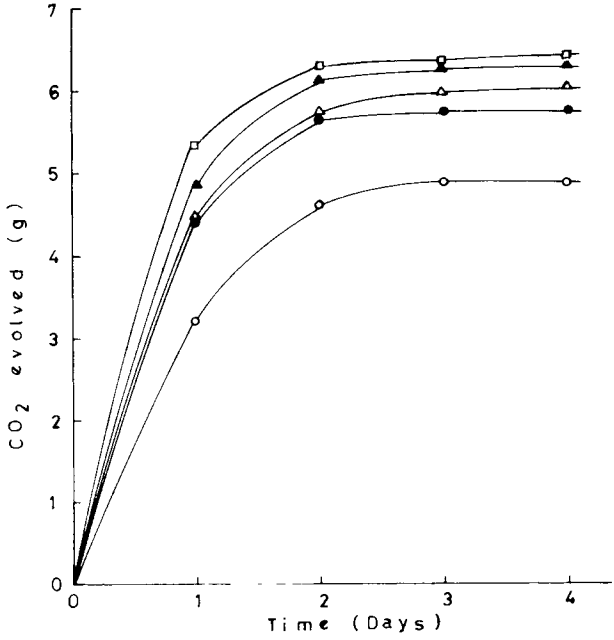

Fig. 4. Relationship between glucoamylase concentration and alcohol yield. Total sugar content of sweet potato is 13.78 g: (○) 100 units, (●) 200 units, (△) 500 units, (▲) 1000 units, and (□) 2000 units.

Alcoholic fermentation was carried out by the procedure described in the Materials and Methods section at three different temperatures: 30, 35, and 38°C. As shown in Figure 3, incubation at 38°C would give the best alcohol yield in three to four days. The effect of various quantities of *E. fibuligera* glucoamylase preparation on alcoholic fermentation at 38°C was tested by varying only the enzyme quantity; this result is shown in Figure 4. The more enzyme added, the higher yield of ethanol was obtained. When 100 units of enzyme was used, the yield was only 70%; but, in the case of 1000 or 2000 units, the yield was about 95%. Yeast concentration also affected the alcohol yield (Table III). The yield of alcohol was found to increase with

TABLE III
Alcoholic Fermentation with Various Yeast (Seed) Concentrations

Yeast (g)	pH after fermentation	Acidity (mL)	Total sugar: Before fermentation (g)	Total sugar: After fermentation (g)	CO_2 formed (g)	Alcohol formed[a] (g)	Alcohol yield[b] (%)
1.0	4.0	4.8	14.07	0.284	5.95	6.28	86.5
2.0	4.0	5.2	14.07	0.255	6.16	6.59	89.6
3.0	4.1	5.2	14.07	0.247	6.40	6.82	93.1

[a]Obtained after distillation.
[b]Calculated from the theoretical ethanol production from total sugar and that from CO_2 evolved.

TABLE IV
Relationship between Amounts of Cellulosine HC and Ethanol Yields

Cellulosine (mg)	CO_2 formed[a] (g)	Alcohol yield (%)
0	3.14	50.2
25	4.71	75.3
50	5.70	91.1
100	5.82	93.0
500	5.82	93.0

[a]Total sugar before fermentation was 12.8 g.

the increase of yeast (seed) concentration. When 1 g of compressed baker's yeast was added, the yield of alcohol was about 86.5%. With 3 g yeast, the yield reached to 93%. Cellulosine HC was found to be essential for the alcoholic fermentation of raw sweet potato since the alcohol yield without it was very low (Table IV). Cellulosine decreased the viscosity of the broth and this might help glucoamylase to attack starch more easily; 100 mg was found to be enough for this purpose. From the results thus obtained, a suitable fermentation condition was the one as described in the Materials and Methods section.

It is clear that alcoholic fermentation of raw sweet potato can be effectively performed by using *E. fibuligera* glucoamylase preparation and 0.05% potassium pyrosulfite for contamination protection. Potassium pyrosulfite is very economical and we anticipate a practical application of this process of alcoholic fermentation of starchy materials without cooking in the industrial scale.

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